

WHAT IS CLAIMED IS:

1           1.     A method for determining whether blood in a stool came from an upper  
2     gastrointestinal site or a lower gastrointestinal site, comprising the steps of:

- 3                   (a)     collecting a stool sample and preparing it for analysis by spectroscopy;
- 4                   (b)     determining a sample absorption spectra of the stool sample; and
- 5                   (c)     determining whether the blood in the stool came from the upper  
6     gastrointestinal site for the lower gastrointestinal site based on an analysis of the sample  
7     absorption spectra.

1           2.     The method of claim 1 wherein step (e) includes performing the analysis to  
2     determine if absorption peaks of the sample absorption spectra are present at approximately  
3     540 nanometers and 576 nanometers, and determining that the blood in the stool sample  
4     came from the upper gastrointestinal tract if the absorption peaks are not present.

1           3.     The method of claim 1 wherein step (e) includes performing the analysis to  
2 determine if an absorption peak of a main Soret band of the sample absorption spectra is  
3 closer to approximately 408 nanometers than to 415 nanometers, and determining that the  
4 blood in the stool sample came from the upper gastrointestinal tract if the main Soret band is  
5 closer to 408 nanometers.

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5 (b) separating the sample stool suspension into a particulate matter portion and a  
6 liquid portion to create a fecal extract;

7 (c) filtering an amount of the fecal extract through a sample filter causing  
8 hemoglobin and related molecules present in the fecal extract to adhere to the sample filter;

9 (d) determining a sample absorption spectra of the sample filter relative to an  
10 absorption spectra of a reference filter that has not been exposed to the fecal extract using a  
11 spectrophotometer; and

12 (e) determining whether the blood in the stool came from the upper  
13 gastrointestinal site for the lower gastrointestinal site based on a analysis of the sample  
14 absorption spectra.

1 6. The method of claim 5 wherein step (e) includes performing the analysis to  
2 determine if absorption peaks of the sample absorption spectra are present at approximately  
3 540 nanometers and 576 nanometers, and determining that the blood in the stool sample  
4 came from the upper gastrointestinal tract if the absorption peaks are not present.

1           7.     The method of claim 5 wherein step (e) includes performing the analysis to  
2 determine if an absorption peak of a main Soret band of the sample absorption spectra is  
3 closer to approximately 408 nanometers than to 415 nanometers, and determining that the  
4 blood in the stool sample came from the upper gastrointestinal tract if the main Soret band is  
5 closer to 408 nanometers.

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(b) separating the sample stool suspension into a particulate matter portion and a liquid portion to create a fecal extract;

(c) filtering an amount of the fecal extract through a sample nitrocellulose filter causing hemoglobin and related molecules present in the fecal extract to adhere to the sample nitrocellulose filter;

(d) determining a sample absorption spectra of the sample nitrocellulose filter relative to an identical reference nitrocellulose filter that has not been exposed to the fecal extract using a spectrophotometer; and

(e) classifying the type of gastrointestinal bleed based on a mathematical analysis of the sample absorption spectra.

10. The method of claim 9 wherein step (e) includes performing the analysis to determine if absorption peaks of the sample absorption spectra are present at approximately 540 nanometers and 576 nanometers, and determining that the blood in the stool sample came from the upper gastrointestinal tract if the absorption peaks are not present.

1            11.    The method of claim 9 wherein step (e) includes performing the analysis to  
2 determine if an absorption peak of a main Soret band of the sample absorption spectra is  
3 closer to approximately 408 nanometers than to 415 nanometers, and determining that the  
4 blood in the stool sample came from the upper gastrointestinal tract if the main Soret band is  
5 closer to 408 nanometers.

1            12.    The method of claim 9 wherein step (e) includes performing the analysis to  
2 determine if absorption peaks of the sample absorption spectra are present at approximately  
3 540 nanometers and 576 nanometers and if an absorption peak of a main Soret band of the  
4 sample absorption spectra is closer to approximately 408 nanometers than to 415 nanometers,  
5 and determining that the blood in the stool sample came from the upper gastrointestinal tract  
6 if the absorption peaks are not present and if the main Soret band is closer to 408 nanometers.

1            13.    The method of claim 9 wherein the sample buffer belongs to a group of  
2 aqueous hypotonic buffers that includes TE buffer comprised of 0.01M  
3 Tris[Hydroxymethyl]aminomethane, 0.001 M Ethylenediaminetetraacetic acid adjusted to pH  
4 7.4.

1           14. The method of claim 9 wherein the stool particulate matter is separated from  
2 the liquid phase by centrifugation and the resulting supernatant fraction becomes the fecal  
3 extract.

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1           15. The method of claim 9 wherein the stool particulate matter is separated from  
2 the liquid portion using a sample cassette, where the stool suspension is passed through a  
3 removable particulate barrier allowing the fecal extract to pass through the sample  
4 nitrocellulose filter and deposit the hemoglobin and related molecules onto the sample  
5 nitrocellulose filter.

1           16. The method of claim 9 wherein the sample nitrocellulose filter and the  
2 reference nitrocellulose are wetted with a 60% glycerol by volume sample buffer to increase  
3 the translucency of the nitrocellulose sample filter aiding in the acquisition of the sample  
4 absorption spectra.

17. The method of claim 9 wherein the mathematical analysis of the sample absorption spectra is accomplished by use of a trained artificial neural network running on a computing device.

18. The method of claim 9 wherein the mathematical analysis of the sample absorption spectra is a Simplex method implemented on a processor and using coefficients obtained from standard spectra for ferrohemoglobin, ferrihemoglobin, urobilinogen and fecal supernatant to maximize the function:

$$Z = \epsilon_{1,\lambda 420}x_1 + \epsilon_{2,\lambda 420}x_2 + \epsilon_{3,\lambda 420}x_3 + \epsilon_{4,\lambda 420}x_4$$

where  $\epsilon$  is the absorption coefficient for the indicated component (1-4) at the indicated wavelength ( $\lambda$ ) obtained from the standard spectra, and  $x$  is the number of units of the indicated component (where component 1 is ferrohemes, component 2 is ferrihemes, component 3 is fecal supernatant, and component 4 is urobilinogen) and subject to the following constraining equations:

$$A_{\lambda 412}/\epsilon_{2,\lambda 412} = \epsilon_{1,\lambda 412}x_1 + \epsilon_{2,\lambda 12}x_2$$

$$0 \geq x_4 - x_3$$

$$A_{\lambda 412} = \epsilon_{1,\lambda 412}x_1 + \epsilon_{2,\lambda 412}x_2 + \epsilon_{3,\lambda 412}x_3 + \epsilon_{4,\lambda 412}x_4$$

$$A_{\lambda 440} = \epsilon_{1,\lambda 440}x_1 + \epsilon_{2,\lambda 440}x_2 + \epsilon_{3,\lambda 440}x_3 + \epsilon_{4,\lambda 440}x_4$$

$$A_{\lambda 494} = \epsilon_{1,\lambda 494}x_1 + \epsilon_{2,\lambda 494}x_2 + \epsilon_{3,\lambda 494}x_3 + \epsilon_{4,\lambda 494}x_4$$

$$A_{\lambda 475} = \epsilon_{1,\lambda 475}x_1 + \epsilon_{2,\lambda 475}x_2 + \epsilon_{3,\lambda 475}x_3 + \epsilon_{4,\lambda 475}x_4$$





1           22.   The method of claim 9 wherein the sample absorption spectrum is obtained  
2 over the range of 400 to 600 nanometers.

1           23.   The method of claim 9 wherein the sample spectra are obtained from ranges of  
2 light wavelengths including infra-red wavelengths and a small number of discrete  
3 wavelengths.

1           24.   The method of claim 23 wherein the amount of ferriheme and ferroheme  
2 present in the fecal extract is determined by one of an infra-red spectroscopy method and a  
3 Fourier transform infra-red spectroscopy (FTIR) method.

1           25.   A cassette system for use in determining whether blood in a stool came from  
2 an upper gastrointestinal site or a lower gastrointestinal site, comprising:

3 (a) a cassette having a volume containing absorbent material, and the top  
4 surface having a first opening;

5 (b) a sample cup/filter device for placement in the first opening, the  
6 sample cup/filter device having a bottom opening and a sample filter covering the bottom  
7 opening,

8 fecal extract in the sample/filter cup device passing through the sample filter  
9 by capillary action aided by the absorbent material to cause hemoglobin and related  
10 molecules present in the fecal extract to adhere to the sample filter.

1 26. The cassette system of claim 25 wherein top surface of the cassette has a  
2 second opening, and includes the reference cup/filter device for placement in the second  
3 opening, the reference cup/filter device having a bottom opening covered by a reference  
4 filter.

1 27. The cassette system of claim 26 wherein the sample filter and the reference  
2 filter are nitrocellulose.

1           28.     The cassette system of claim 25 wherein the cassette includes a connection  
2     port for connection to a vacuum source for providing a vacuum in the cassette to assist the  
3     capillary action.

1           29.           The cassette system of claim 25 wherein the absorbent material  
2     includes absorbent paper.

FIG. 2 is a cross-sectional view of the cassette system of claim 25.